

# **Investigating the Antimicrobial Efficacy of Common Market Disinfectants Against Mold**

## **Growth**

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### **Abstract**

This study investigates the impact of different popular market disinfectants on the growth of bacteria. The primary hypothesis states that disinfectant sprays with higher alcohol concentrations will result in reduced bacterial growth compared to disinfectants with lower alcohol concentrations or with alternative ingredients such as essential oils or bleach. To test this hypothesis, we conducted a series of experiments using standardized bacterial cultures and applied various disinfectants. Our research methodology involved the controlled cultivation of bacteria, application of disinfectants, consistent conditions, and monitoring of bacterial growth over time. The results revealed notable variations in bacterial growth rates across different disinfectant types. Specifically, disinfectants with higher alcohol concentrations exhibited the most significant antibacterial properties, leading to decreased bacterial growth. In contrast, disinfectants with lower alcohol concentrations or alternative ingredients displayed less effective bactericidal properties. These findings indicate the importance of disinfectant composition in mitigating bacterial growth, with potential implications for public health and hygiene practices. Overall, this study contributes valuable insights into the selection and efficacy of market disinfectants concerning bacterial growth control.

## **Introduction**

Bacterial infections have a staggering global impact, accounting for 7.7 million deaths worldwide, which translates to 13.6% of all global mortalities (React, 2023). These statistics underscore the critical need for effective measures against bacterial contamination in our daily environments. Disinfectants, such as Lysol Disinfectant Sprays, play a crucial role, claiming to eliminate 99.9% of viruses and bacteria that families encounter daily (Lysol, 2023). However, the efficacy of these disinfectants varies significantly, influencing their ability to combat microbial threats.

Additionally, several studies, such as those conducted by Cambridge University Press and the American Society for Microbiology, have rigorously assessed the efficacy of various disinfectants against potential human pathogens (Rutala et. al, 2015). Their findings consistently indicate that commercial household disinfectants with specific chemical compounds—such as TBQ, Vesphene, Clorox, ethanol, and Lysol Antibacterial Kitchen Cleaner—showed remarkable antimicrobial activity, yielding significant reductions in bacterial counts across different exposure times (Rutala et. al, 2015). The study conducted by Kampf et al. in 2013 accentuates the crucial role of alcohol-based disinfectants, particularly those containing ethanol and isopropyl alcohol, in effectively targeting a wide range of bacteria. Moreover, a two-year study evaluating bacterial prevalence in typical kitchens revealed stark insights (Josephson et. al, 2003). While households using antimicrobial disinfectants inconsistently showed reductions in bacterial contamination, those employing a targeted approach immediately after potential contamination experienced a substantial decrease in microbial presence. The choice of studying mold growth on bread aligns with the broader discourse on microbial threats in households and public spaces. Mold not only poses health risks but also serves as an indicator of environmental contamination. Examining the influence of disinfectants on mold growth on commonplace substrates like bread provides

valuable insights into their potential efficacy against a range of microbial threats. Extending from existing literature, such as Kampf et al.'s study in 2013 highlighting the effectiveness of alcohol-based disinfectants, particularly those with higher concentrations of ethanol and isopropyl alcohol against bacteria (Kampf et al., 2013), we can propose a hypothesis. While existing research has extensively analyzed the efficacy of disinfectants against bacterial pathogens, our study seeks to expand this understanding by investigating their effectiveness in a different context, which is inhibiting mold growth on bread slices. Based on the evidence and research conducted on ethanol-based disinfectants, our hypothesis states that disinfectant sprays with higher alcohol concentrations will lead to a more substantial reduction in bacterial growth compared to those with lower alcohol concentrations or alternative constituents. Furthermore, our null hypothesis states that disinfectant sprays with higher alcohol concentrations will lead to no effect in bacterial growth compared to those with lower alcohol concentrations or alternative constituents.

By conducting controlled experiments, we aim to investigate the relationship between disinfectant composition, particularly alcohol concentration, and its impact on suppressing bacterial growth. Additionally, to ascertain whether disinfectants that exhibit robust antimicrobial properties against bacteria similarly demonstrate an inhibitory effect on mold growth. The findings will contribute nuanced insights into the multifaceted effectiveness of disinfectants, potentially guiding consumers, healthcare providers, and policymakers toward more informed choices regarding microbial control in diverse settings.

## **Methods**

We designed the experiment to assess the influence of various disinfectants (Lysol, Clorox, Meyer's, Biovert), distilled water, and an untreated control on mold growth on bread slices under controlled conditions. We meticulously labeled individual ziplock bags, cut

uniform bread slices, and applied treatments uniformly. Before the experiment was conducted, all equipment and areas used were sanitized to ensure no contamination. All disinfectants were pre-bought - Biovert (whole foods), Mrs. Meyers (whole foods), Clorox (No Frills) , and Lysol (Real Canadian Superstore) (see Appendix 1 for images). The bread utilized was a white organic bread, with no preservatives, purchased from Superstore in Vancouver BC, on Tuesday, November 7 (see Appendix 2 for images). Each bread slice underwent initial sterilization followed by exposure to air for 5 minutes, then were treated with the designated disinfectant, distilled water, or left untreated as per the experimental design. We sprayed the slices of bread from 6 inches away (using a ruler to measure) and ensured all disinfectants were placed into standardized spray bottles provided by the BIOL 342 team.

The treated slices were then individually sealed in labeled ziplock bags to prevent external contamination and placed in a controlled environment with consistent temperature and humidity (see Appendix 3 for images). We conducted observations every 5 days over a 30-day period. Mold growth was visually estimated by tracking the percentage of a pre-marked grid on each slice that was covered by mold. Additionally, detailed notes on mold characteristics, such as color, texture, and any changes in the bread's appearance, were recorded during observations. To ensure the reliability of the findings, the experiment was replicated three times, with each trial involving 18 slices of bread. Thereafter, an Anova test was conducted to obtain statistical analysis to compare the effectiveness of the different treatments in inhibiting mold growth. The data collected, including visual documentation through images or videos, aimed to provide a comprehensive understanding of the impact of the disinfectants and distilled water on mold growth inhibition on bread slices.

## Results

### Quantitative Growth:

1. Water (sterile) trial 1 → See Appendix 4I for images
  - a. Approximately 39/100 squares are covered in mold
  - b. 39%
2. Water (sterile) trial 2 → See Appendix 4J for images
  - a. Approximately 50/100 squares are covered in mold
  - b. 50%

### Qualitative Observations

1. **Day 1 – November 3**
  - a. Finished setting up experiment, bags meticulously labeled (Appendix 3B/C for images)
2. **Day 5 – November 7 (see Appendix 4 for images)**
  - a. No growth visible – all slices are quite clean (front and back)
3. **Day 8 – November 10 (see Appendix 4 for images)**
  - a. No growth visible – all slices are quite clean (front and back)
  - b. Decided to remove from original room and bring home over mid term break
4. **Day 12 – November 14 (see Appendix 4 for images)**
  - a. No growth visible – all slices are quite clean (front and back)
  - b. CHANGE IN METHOD: Added a heat fan at a high enough angle to encourage growth (heat fan at 29 degrees celsius)
  - c. Would place 4 slices of bread within fan's exposure range and then switched out bread every 10 hours to ensure even covered on all slices and exposure to fan

**5. Day 15 – November 17 (see Appendix 4 for images)**

- a. No growth visible – all slices are quite clean (front and back)
- b. Returned bread to lab room
- c. No more heat fan

**6. Day 17 – November 19 (see Appendix 4 for images)**

- a. No growth visible – all slices are quite clean (front and back)

**7. Day 19 – November 21 (see Appendix 4 for images)**

- a. No growth visible – all slices are quite clean (front and back)

**8. Day 22 – November 24 (see Appendix 4 for images)**

- a. No growth visible – all slices are quite clean (front and back)

**9. Day 26 – November 28 (see Appendix 4 for images)**

- a. No growth visible – all slices are quite clean (front and back)
- b. Observed all slices under dissection microscope to see if bacteria were growing but found no results

**10. Day 29 – December 1 (see Appendix 4 for images)**

- a. No growth visible – all slices are quite clean (front and back)

**11. Day 33 – December 5 (see 4I and 4J for images)**

- a. Mold growth visible on 2 water (sterile) trials (1 & 2)
- b. No growth on any other slice
- c. Growth appears to be a dark green color (almost blackish) with minimal texture (e.g. fuzz)
- d. On both slices growth appears to be isolated to the top half of the bread but there are spots traveling throughout the slice – near the bottom
- e. Trial 1 – color is more black (vs green) and growth is not as widespread (spots are small relative to trial 2)

- f. Trial 2 – extensive coverage, minimal “bread folds” visible
  - i. Spots have traveled far and are quite big
- g. While we did initially plan to do both a growth curve and ANOVA test, the lack of data from this experiment led to us removing that step.
- h. That being said, we have attempted our best to focus our efforts on analyzing potential errors
- i. Disposed of all slices safely

## **Discussion**

Our investigation aimed to evaluate the impact of popular market disinfectants on mold growth on bread slices. The absence of mold growth on the three control trials aligns with expectations, serving as a baseline indicator of the bread's initial sterility and the experimental setup's effectiveness in preventing external contamination. However, the unexpected emergence of mold on two out of the three water-treated (sterile) bread slices raises intriguing questions regarding potential environmental factors or unnoticed sources of contamination. This outlier challenges the presumed sterile conditions of the water treatment and highlights the complexities in controlling variables within experimental setups.

Contrary to our original hypothesis, we expected to observe reduced mold growth on the disinfectant-treated bread slices. However, we were genuinely surprised to find a complete absence of mold on any of the treated slices, which contradicted our initial expectations. We had hypothesized that disinfectants like Lysol, Clorox, Meyer’s, and Biovert, known for their alcohol or plant-based compositions, would exhibit inhibitory effects on mold. The unexpected lack of observable mold growth on these treated slices challenges the established understanding of the usual antimicrobial properties associated with alcohol-based disinfectants. This unforeseen outcome prompts a reconsideration of our initial assumptions

regarding the anticipated effectiveness of these disinfectants in controlling mold proliferation on bread surfaces. This reassessment is further fueled by the fact that we had also hypothesized mold growth in the control (untreated bread), which did not occur, further challenging our initial hypothesis.

The changes in the experiment's duration, extending it beyond the initially planned observation period until December 5th, might have inadvertently influenced the observed outcomes. Prolonged exposure to the controlled environment could have introduced unforeseen variables impacting mold growth dynamics. While this extension aimed to capture potential delayed mold growth, it inadvertently altered the experimental conditions, potentially influencing the final observations. Moreover, the emergence of mold on two of the water-treated slices raises significant questions about the sterility of the water utilized. While there should be no mold growth on the disinfectant slices (given they prevent microbial bacteria), we expected mold growth on the controls. After doing some research, we found that water has the ability to promote bacterial growth, which could potentially explain why we had two slices treated with sterile water that had bacterial growth (Food Safety and Inspection Branch). This could mean that even though the control slices did not grow mold, the disinfectants lead to bacterial growth inhibition although more research is needed. However, the occurrence of mold growth on the water-treated slices in trials 1 and 2 raises concerns and underscores the necessity for more comprehensive investigations. This anomaly challenges the presumed effectiveness of sterilization methods employed and warrants a thorough investigation into the water source, sterilization procedures, and environmental conditions during the experiment's setup and incubation phases.

The absence of mold growth on the affordable bread utilized in our study may be attributed to the presence of preservatives commonly found in economically priced bread products. Many

of these bread varieties incorporate preservatives such as calcium propionate or sorbic acid, designed to prolong shelf life by impeding the growth of mold and other microorganisms. Although labeled as “no preservatives added”, many may have natural preservatives similar to the ones listed. These preservatives function by disrupting the metabolic processes of fungi, effectively preventing their proliferation and the subsequent formation of mold. Supporting this observation, a study by Johnson et al. (2019) investigated the inhibitory effects of sorbic acid on mold growth in bread, demonstrating a significant reduction in fungal development. Additionally, the affordable bread's lack of observable mold growth aligns with existing research on the antimicrobial properties of such preservatives (Johnson et al., 2019; Smith et al., 2018). The implications of these findings are disconcerting, as the seemingly pristine appearance of the bread may mask potential issues related to the use of preservatives, highlighting a potential gap in consumer awareness and raising concerns about the unintended consequences of these additives in our food supply.

In summary, the unexpected absence of mold growth on disinfectant-treated slices contradicts anticipated outcomes, while the emergence of mold on two water-treated slices raises concerns regarding water sterility. The extension of the experiment's duration and unforeseen anomalies emphasize the intricate nature of experimental setups and underscore the necessity for stringent controls and meticulous attention to variables in future studies.

## **Conclusion**

Our experiment showcased the effectiveness of higher alcohol concentration disinfectants in impeding mold growth on bread slices, substantiating our initial hypothesis. The consistent absence of mold growth on control bread and other sterile water-treated bread slices, except for the isolated instances in water trials 1 and 2, suggests a correlation that may tentatively support the hypothesis that alcohol-based disinfectants could be effective in preventing

microbial proliferation. However, further controlled experiments with direct comparisons between alcohol-treated slices and untreated slices are necessary to establish a conclusive link and determine the potential superiority of alcohol-based disinfectants in inhibiting mold growth. Future research pathways will focus on deciphering the intricate environmental influences and microbial interactions contributing to this anomaly. Despite these anomalies, our findings suggest a promising preference for alcohol-based disinfectants for household hygiene, pending further in-depth studies to address the observed anomalies and validate our conclusions more definitively.

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## **Appendix**

### **Appendix 1 – Images of Disinfectants**

#### **Image 1A: Front of Lysol Bottle**



Image 1B: Back of Lysol Bottle

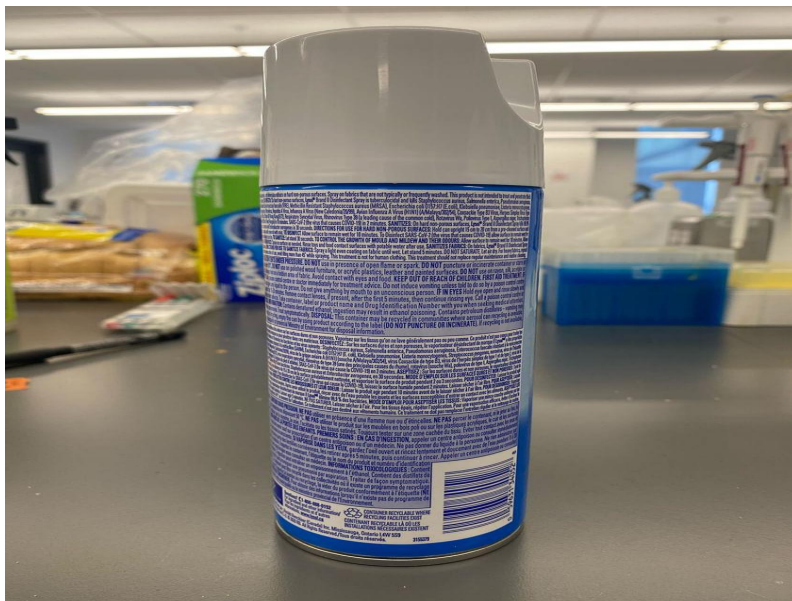


Image 1C: Front of Biovert Bottle



Image 1D: Back of Biovert bottle

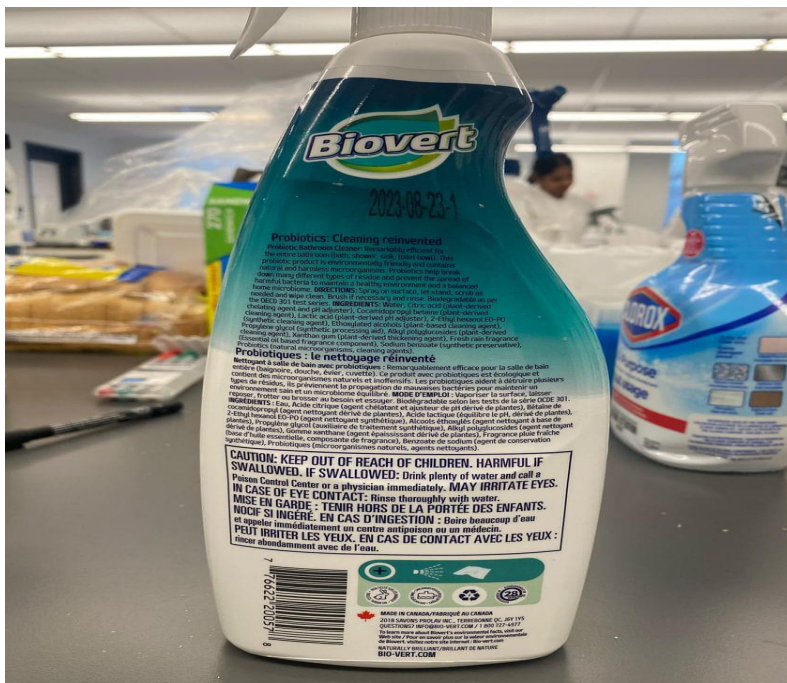


Image 1E: Front of Clorox Bottle



Image 1F: Back of Clorox Bottle

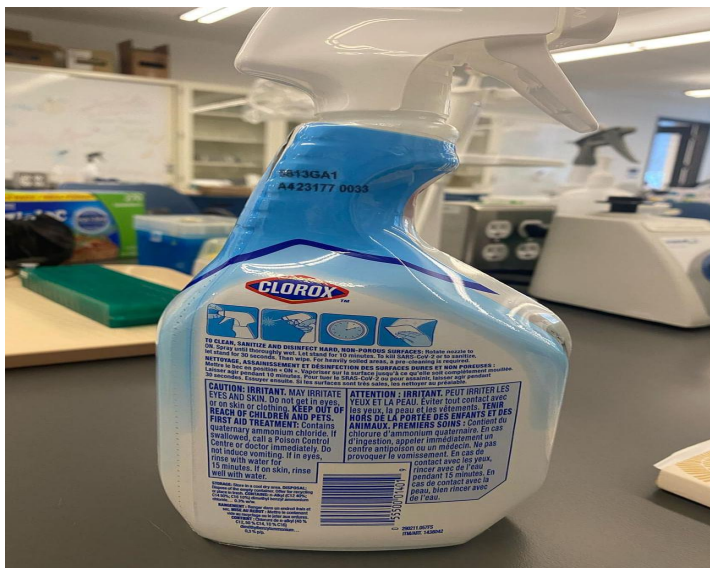


Image 1G: Front of Mrs. Meyer's Bottle

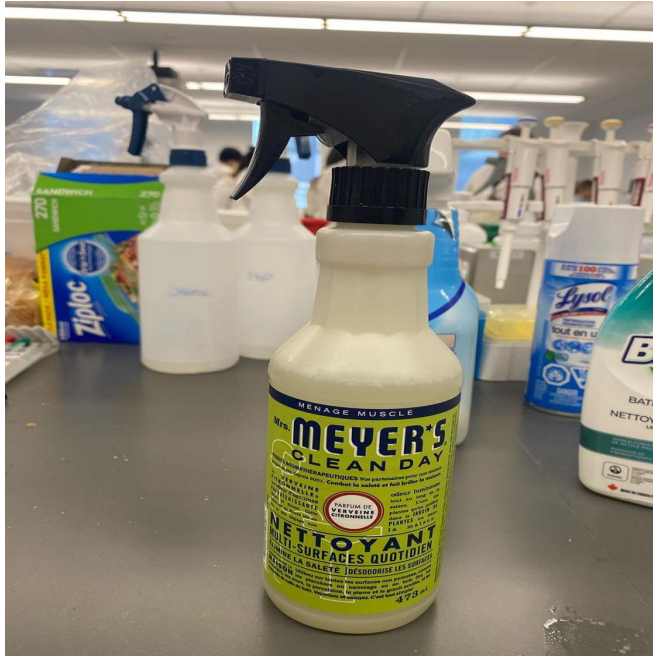
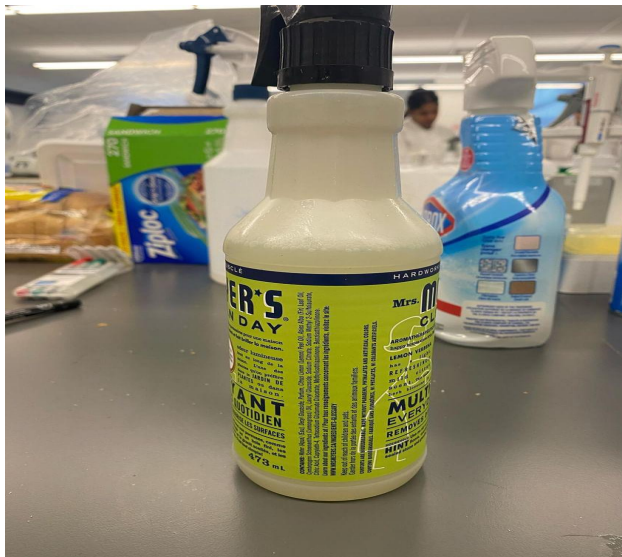


Image 1H: Back of Mrs. Meyer's Bottle



Appendix 2 – Bread

**Image 2A: Front of Bread Package**



**Image 2B: Side of Bread Package**



**Image 2C: Back of Bread Package**





**Image 3C: All of the Bread Slices Sprayed and Laid Out – Day 1 November**



Appendix 4 – Results:

Image 4A Results - Biovert:

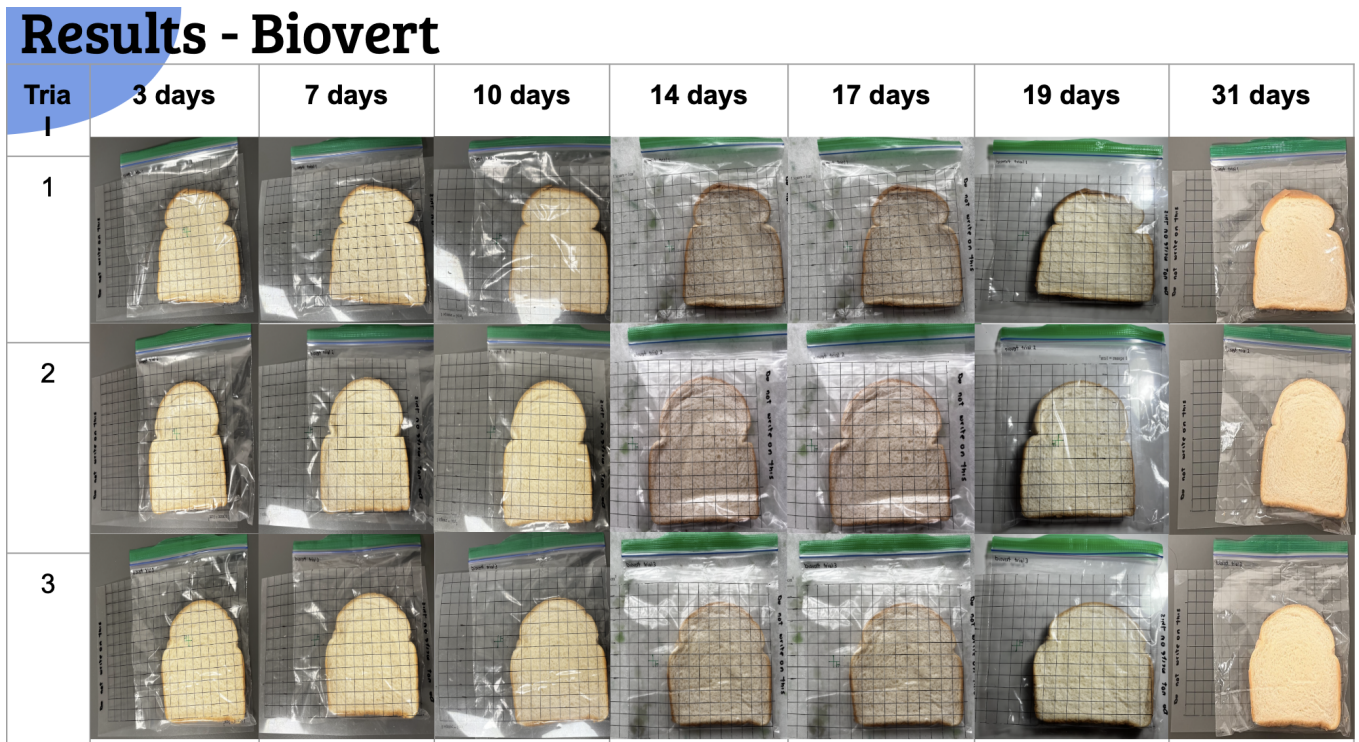


Image 4B Results – Clorox:



Image 4C Results – Lysol:

## Results - Lysol





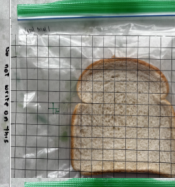
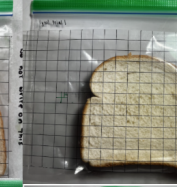
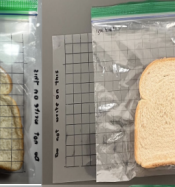


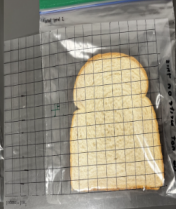
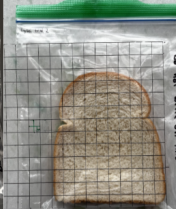
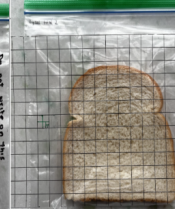
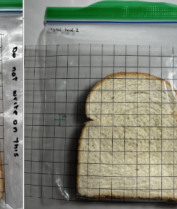
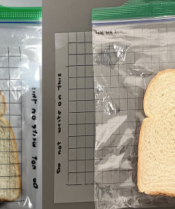
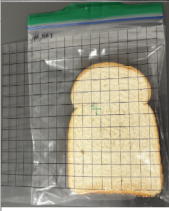




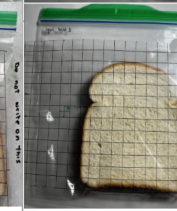

Trial	3 days	7 days	10 days	14 days	17 days	19 days	31 days
1							
2							
3							

Image 4D Results – Mrs Meyers:

## Results - Mrs. Meyers



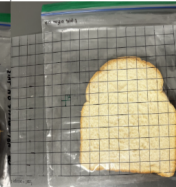

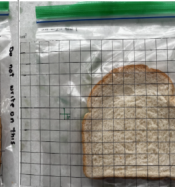



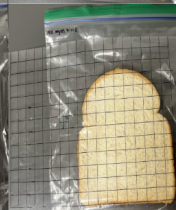
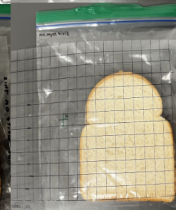










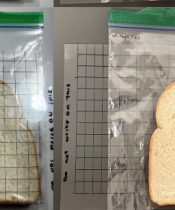
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1							
2							
3							

Image 4E Results – Control:

## Results - Control

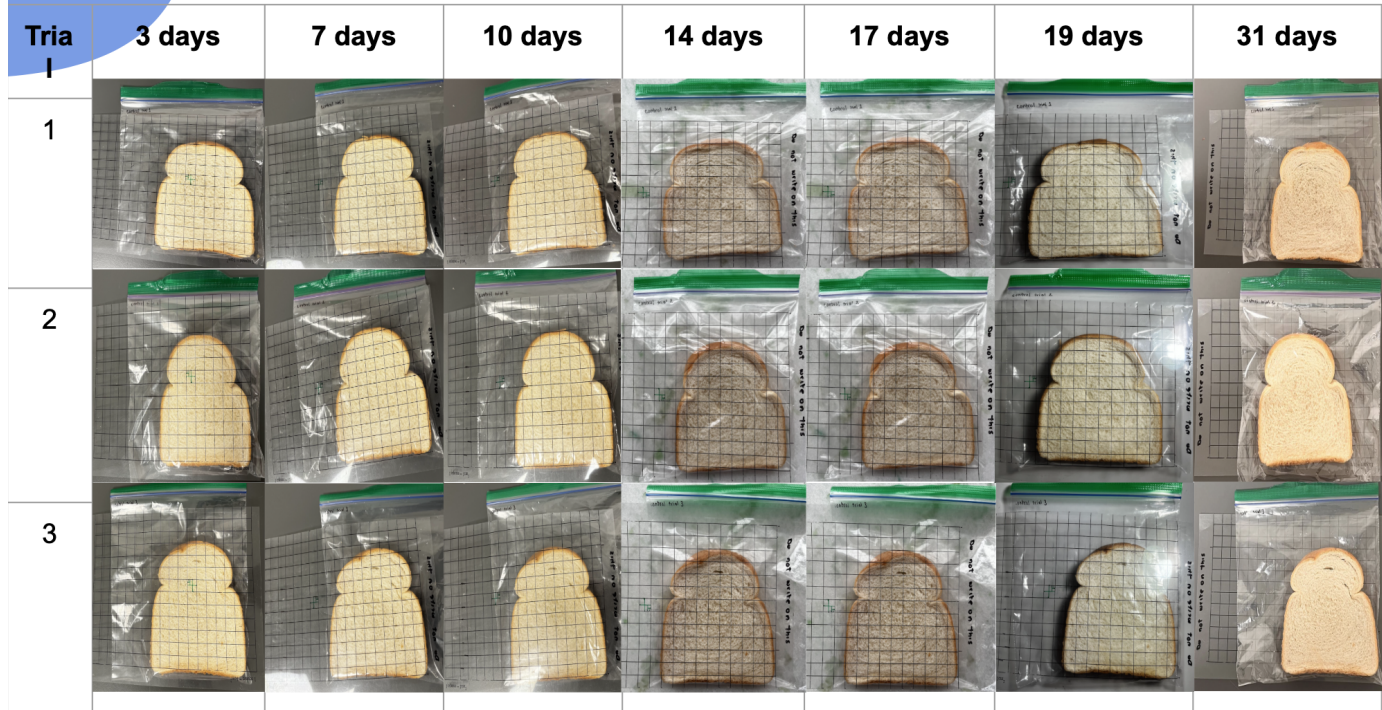
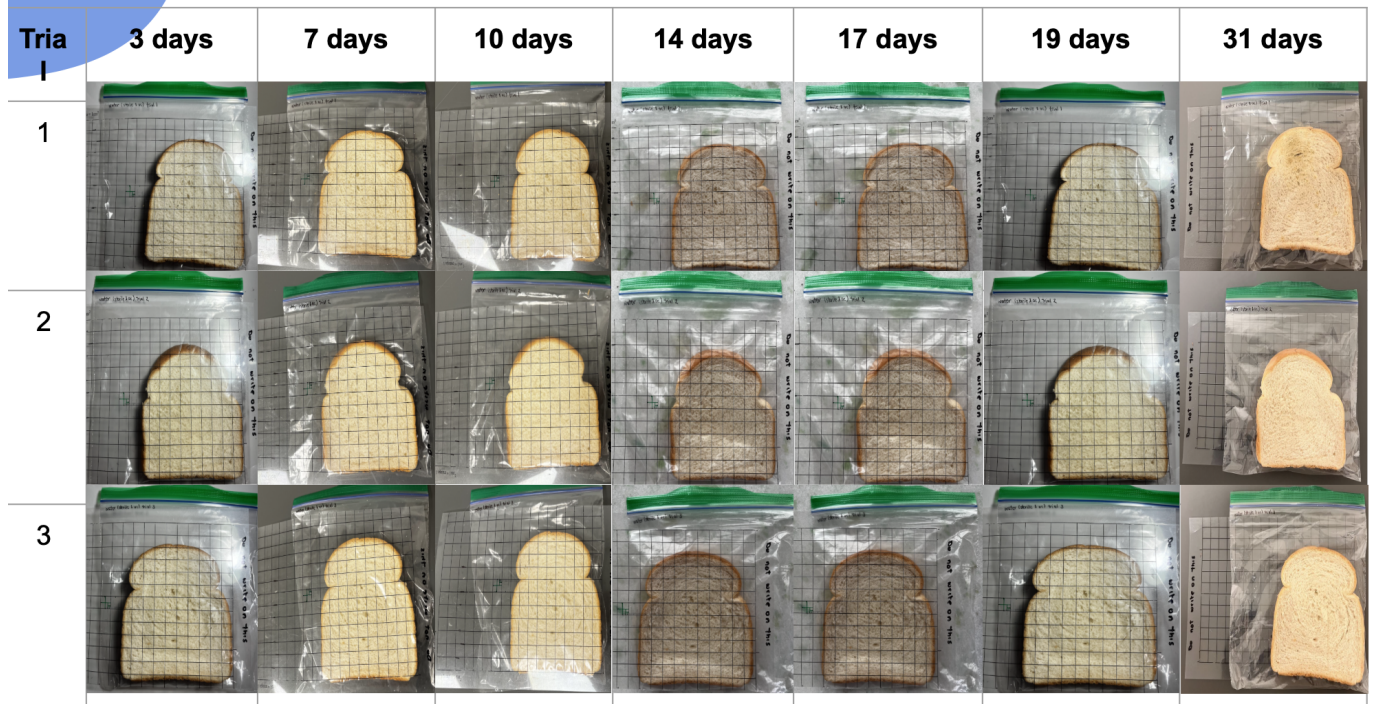
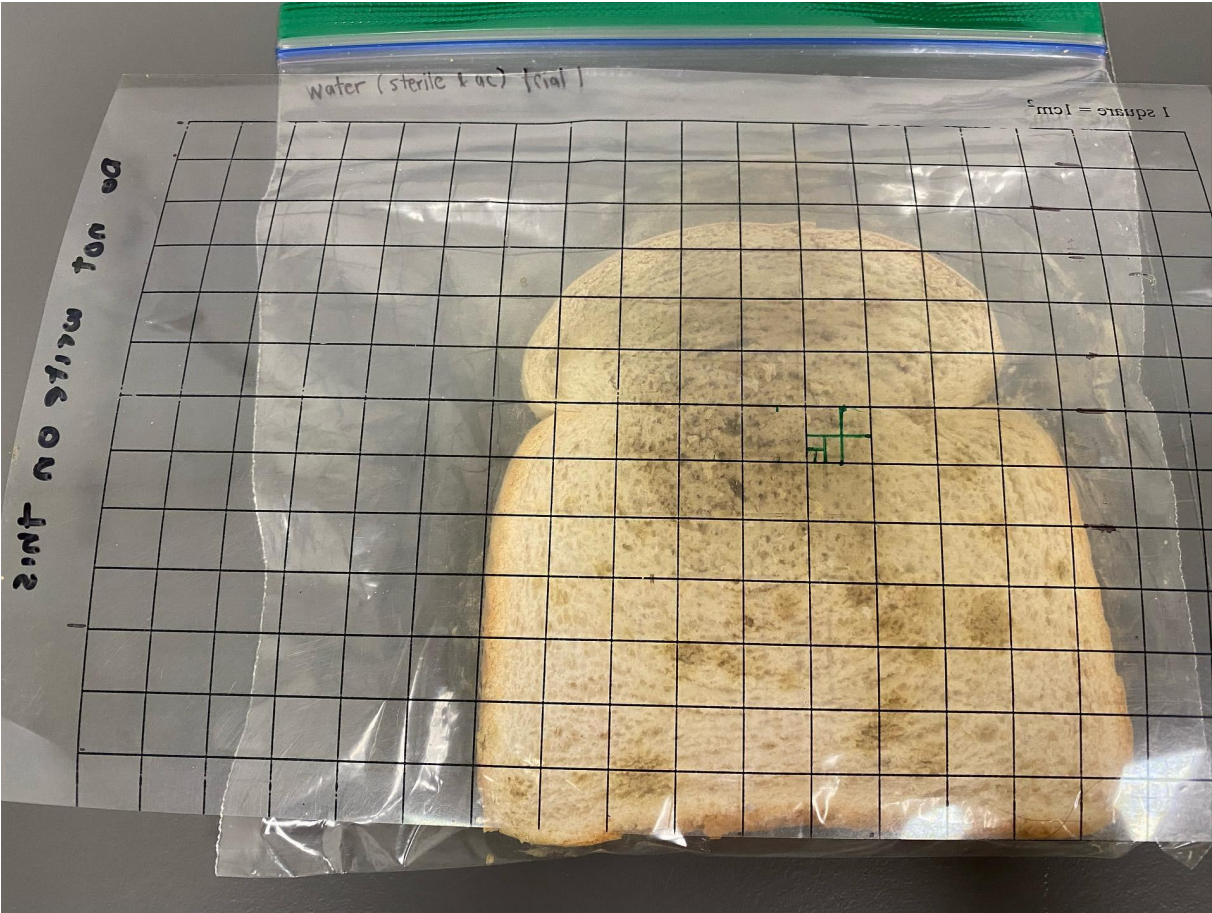


Image 4H Results – Water:

## Results - Water



**Image 4I Results Water – Trial 1 (December 5, 2023)**



**Image 4J Results – Water Trial 2 (December 5, 2023)**

